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(54) CALF FEED CONTAINING SORANGIUM ENZYMES

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ABSTRACT

Inclusion of a protease material in the feed of young calves renders non-milk protein digestible and allows the calves to be raised on milk replacers in which expensive milk protein is replaced by cheaper plant protein. The protease employed is an extra-cellular metabolite of a Sorangium sp.(isolate 495).

This invention relates to a novel feed composition suitable for feeding to young calves, and more particularly to such a compositon which comprises a milk replacer.

It has been a common practice for many years to raise calves by means other than allowing them to nurse their mothers. Normally, a young calf is completely dependent upon its mother,s milk for its nutritional requirements for a period up to 42 days after its birth, until such time as it is able to obtain all the nutrients required for normal and healthy growth from other sources, particularly plant matter. According to conventional methods of raising calves apart from their mothers, calves are fed after weaning (i.e. after the first three to four days of their lives) and for a period up to and until they are about seven weeks old on a so-called "milk replacer".

The conventional milk replacers consist mainly of milk products in the form of skim milk and whey together with oils, fats, vitamins and trace minerals giving a balanced blend of proteins, fat, carbohydrates, vitamins and minerals meeting the calves' nutritional requirements.

The major problem with conventional milk replacers is one of cost. The high, and rising, costs of milk products make conventional milk replacers expensive, and result in higher costs for raising calves, which are reflected in higher meat prices to the purchasers of meat products and high costs of raising replacement stock.

The high costs associated with the use of conventional milk replacers containing milk protein have led to the consideration of the inclusion of non-milk protein in milk

replacers. By blending together non-milk proteins, e.g. proteinaceous plant materials, in the appropriate amounts, and with the addition of synthetic essential amino acids if necessary, it is possible to obtain a mixture containing the essential amino acids in proportions substantially corresponding to those of milk protein, and which could theoretically be substituted, at least partially, for milk protein in milk replacers. However, replacement of any substantial amount of milk protein source by a non-milk protein source has, to date, been unsuccessful.

For the early part of a calf's life, its rumen is insufficeintly developed to allow the proper and required digestion of foods containing any substantial amount of non-milk protein. In order to digest plant proteins adequately, a normal flora of bacteria and protozoa needs to be established in the calf's rumen. These bacteria and protozoa then produce and secrete the enzymes which are capable of digesting plant proteins. The development of this flora of bacteria and protozoa in the rumen is a gradual one, and although the calf is usually producing enzymes by about age 2 weeks, the development is only fully completed after the calf reaches the age of 6-8 weeks.

Notwithstanding these problems associated with the use of non-milk protein in milk replacers, attempts have been made to reduce the costs of conventional milk replacers by using a variety of products as substitutes for a portion of the milk protein in the milk replacer. While some manufacturers of milk replacers containing substantial amounts of non-milk protein claim efficient utilization of

of the non-milk protein, it is generally considered that the better quality milk replacers are those consisting entirely of milk protein. The use of low-quality milk replacers, having a higher proportion of non-milk protein, is felt to have contributed to the many nutritional, health and environmental problems associated with the raising of calves, and the resulting deaths of calves represent a serious financial loss to calf raisers. Thus while some initial savings on the cost of the milk replacer may be obtained by replacing some of the milk protein by non-milk protein, these savings are often equalled or exceeded by the losses occurring at the farm level when these products are fed to young calves.

We have now found that young calves may be raised on a milk replacer containing non-milk protein without the aforementioned nutritional, health and environmental problems resulting, and without unacceptable digestive disturbances occurring, if the feed is supplemented with a protease material which is an extracellular metabolite of a Sorangium sp.(isolate 495).

The said Sorangium sp.(isolate 495) is maintained in and is available from the Chemistry and Biology Research Institute, Agriculture - Canada (formerly known as the Microbiology Research Institute, Canada Department of Agriculture), Ottawa. It is described in United States Patent No. 3,515,641 dated June 2, 1970 assigned to Canadian Patents and Development Limited, to which reference should be made for details of procedures for culturing and growing the bacterium. Details of compositions of suitable growth media and of other growth conditions are disclosed in Example 1 of the patent. The protease material is present in

the liquid portion of the fermentation broth.

As a result of taxonomic studies conducted on the said bacterium, we believe it should more properly be classified as Lysobacter enzymogenes. However, since such is the name under which the bacterium is available and under which it is discussed in the literature, we shall refer to it herein as a Sorangium sp.(isolate 495) for the sake of clarity and to avoid confusion.

In the preferred practice of the present invention, we use the whole fermentation broth, or we separate the liquid portion from the solids portion using conventional methods of separation such as centrifugation or filtration, and the supernatant or liquid portion can then be concentrated under vacuum and dried by freeze-drying or spray-drying to yield the protease material in concentrated solid form.

As described in the above-mentioned patent, two distinct enzymes can be isolated from the fermentation broth, and the trivial names α- and β-lytic protease have been assigned to them. These two enzymes are reasponsible for most of the proteolytic activity which is believed to account for the beneficial results obtained with our feed supplement, but since entirely satisfactory results can be obtained employing a crude mixture of the enzymes which are present in the liquid portion of the fermentation broth, there is no need to isolate individual enzymes for the purposes of the present invention. Further, the whole fermentation broth contains many valuable nutrients, so there is little

advantage in separating the protease material in the liquid portion from the cellular portion of the broth.

Employing our protease material feed supplement, we have found that calves can be raised satisfactorily on milk replacers containing high proportions of non-milk protein. By way of example, the content of non-milk protein may be in excess of 50% of the total protein in the milk replacer. The formulation of milk replacers containing substantial contents of mon-milk protein, is well within the capabilities of those skilled in the art, and forms no part of the essence of our invention. It is necessary to note merely that milk protein contains all the amino acids required by the calf and it is desirable that the non-milk protein milk replacer should contain at least the minimum amounts of the amino acids believed necessary for adequate nutrition in appropriately balanced proportions. It can be mentioned by way of example that milk replacers can be formulated from blends of soy protein meal and corn gluten meal, which latter has a high content of the aromatic amino acids and valine that are lacking from soy protein, together with additions of methionine.

In the practice of the present invention, the protease material feed supplement and the milk replacer may be mixed with warm water and fed from a pail or a nipple, generally following the conventional procedures for raising calves on milk replacers. Conventional feed additives, including vitamins, trace minerals and antibiotics, may be included in the feed composition.

Any amount of the protease material added to a milk

replacer containing non-milk protein will assist, to some extent, in the digestion of the non-milk protein. Moreover, a large excess of protease material added to the milk replacer would not be harmful to the calf, but would merely be wasteful, as more of the protease material would be used than would be required to enable the calf to fully digest the non-milk protein.

The optimal amount of protease material to be added to the milk replacer is dependent upon several factors, including the nature and composition of the milk replacer, the proportion of milk protein to non-milk protein in the milk replacer, and the state of development of the digestive system of the individual calves to which the milk replacer is to be fed.

An estimate of the minimum amount of the protease material required can be made in vitro by determining the amount of protease material required to hydrolyze a given amount of the milk replacer in the laboratory. However, the estimate has to take into account the fact that not all the enzyme material will be utilised efficiently, and that some will be destroyed.

In practice, we prefer to employ a somewhat greater quantity of protease material than would optimally seem to be required; a substantial increase in the amount of protease material present would not substantially reduce the savings realized through the use of a non-milk protein in the preparation of the milk replacer.

Generally, we have found that satisfactory results can be obtained when the protease material is present in an

amount exhibiting a protease activity in the range 500 to 3,000 D.U. (Delft Units) per gram of non-milk protein in the milk replacer, and more preferably about 2,000 D.U. per gram of non-milk protein in the milk replacer.

A Delft Unit is an arbitrary unit used to indicate the protease activity of an enzyme preparation, and is determined by following the assay procedure described in the Maxatase* brochure available from Royal Netherlands Fermentation Industries, P.O. Box 1, Delft, Holland. Reference to this brochure should be made for the full details. Briefly, the assay procedure relies on measuring the activity of the enzyme preparation in digesting a standardised casein substrate.

The protease material feed supplement may conveniently be formulated as a unit dosage containing an amount of the protease material exhibiting an appropriate protease activity, which can then be fed to the calves along with the milk replacer.

Advantageously, the unit dosage will contain protease material in an amount exhibiting a protease activity of from 10,000 D.U. to 200,000 D.U. At the time when the protease material supplement has the greatest influence of the calf's digestion of non-milk protein, i.e. during the first two weeks of the calf's life, the calf will typically be receiving about 500 g of milk replacer per day, with a protein content of about 20%, in two equal daily feedings of 250g. With a non-milk protein content of at least 50% in the milk replacer, it would be appropriate to employ a dosage exhibiting at least 12,500 D.U., preferably about

* trade mark

50,000 D.U. protease activity to be added to each feeding. With higher non-milk protein contents, dosages of as much as 200,000 D.U. may be used.

The following non-limiting Examples illustrate the practice of the present invention.

EXAMPLE

Thirty-two Holstein bull calves, purchased at approximately 6 days of age, were randomly allotted on the basis of initial weight and age to a control group and two experimental groups. Two experimental replacers, with 50% (50%P) and 80% (80%P) of the protein in the milk replacer supplied by plant (P) protein, were formulated and compared to a control all-milk protein replacer (0%P). The composition of these milk replacers is shown in Table 1, wherein, as throughout this specification, all parts and percentages are by weight.

Table 1

	0%P	50%P	80%P
Ingredients (%):			
Dried skim milk	40	22.5	11.5
Spray dried whey	10	27.5	13.5
Dried buttermilk	15	-	-
Tallow & cocoanut oil (1)	21	21	21
Dextrose & corn starch (2)	13	13.9	28.4
Promine F (3)	-	8.5	16.0
Corn gluten meal	-	5.5	8.5
Vitamins & Minerals (4)	1	1	1
Methionine	-	0.1	0.1

Table 1 cont.

	0%P	50%P	80%P
Composition (% dry matter basis)			
Protein	20.9	22.4	25.4
Fat	21.0	21.0	21.0
Ca	1.0	1.0	1.0
Phosphorus	0.75	0.75	0.75

(1) Mixed in 3:1 proportion. Fancy grade tallow, supplied by St. Lawrence Rendering Co., Montreal, Que. Unrefined cocoanut oil supplied by Drew Brown Ltd., Montreal, Que.

(2) Mixed in 3:1 proportion. Both supplied by Canada Starch Ltd., Cardinal, Ont.

(3) Isolated soy protein (90%) supplied by Central Soya Co., Inc., Chicago, Illinois.

(4) Commercial mix supplied by Delmar Chemical Ltd., Montreal, Que.

A feed supplement containing protease material was prepared by growing Sorangium sp.(isolate 495) under the conditions described in Example 1 (a) of United States patent 3,515,641. The whole fermentation broth was then concentrated by evaporation, and sufficient of the concentrated broth, as determined by assay of the D.U. per gram, was blended with two lots of soybean meal carrier to form two mixtures, one of higher and one of lower protease material content, which were then divided into 14 g. dosage units. The dosage units of higher protease content possessed 100,000 D.U., and those of lower protease content possessed 50,000 D.U.

The calves were fed the milk replacers allotted to them, the milk replacers being mixed with warm water and being fed from a pail or through a nipple. Each calf was given two equal daily feeds of its allotted milk replacer. The amounts of milk replacer and, of the water to dilute the milk replacer fed to each calf per day were as shown in table 2.

Table 2

Components	Week						Total
	1st	2nd	3rd	4th	5th	6th	
Milk Replacer (g)	500	700	900	900	900	625	31,675
Water (litres)	4	4.5	5.0	6.0	6.0	6.0	220.5

In the case of the first experimental group which received the 50% P milk replacer, one 14g dosage unit of the 50,000 D.U. protease material-containing feed supplement was blended into the milk replacer before feeding, so that each calf in this group received protease material of 100,000 D.U. daily.

The calves in the second experimental group each received one 14g dosage unit of the higher protease content with each feeding of the 80%P milk replacer, so that they received a total of 200,000 D.U. daily.

No protease-containing feed supplement was offered to the calves in the first, or control, group.

For the first three days, each of the calves in all three groups were injected daily with a conventional commercially available vitamin preparation and were offered 50g

of electroyltes (Na Cl, K Cl and Na HCO_3) mixed with dextrose as a carrier.

Calf starter (composed of grouned corn, barley, wheat bran, soybean meal molasses and vitamins and minerals) with a crude protein content of 18%, timothy/trefoil hay, and fresh water were offered to all calves as free choice from day 1.

Attention was paid to balancing the proportions of the essential amino acids in the 50%P and 80%P experimental milk replacers, so as to obtain, as nearly as economically feasible, balanced proportions of amino acids substantially corresponding to those of milk protein.

The essential amino acid contents of the milk replacers employed in the present Example are shown in Table 3, as well as the amino acid contents which are contributed by 28 g. of the protease material-containing feed supplement.

Table 3

Replacer	Grams Protein/ 1000 g Replacer	Essential amino acids, g/1000 g replacer									
		Lysine	Methio-nine	Cystine	Threo-nine	Leucine	Isoleu-cine	Phenyl-alanine	Tyro-nine	Valine	Trypto-phan
0%P*	209.0	16.90	6.80	4.0	10.20	21.50	10.50	11.80	10.90	12.70	2.30
50%P*	223.9	14.56	6.76	4.25	10.10	23.60	10.82	13.23	11.62	10.97	2.69
80%P*	254.0	14.70	6.74	5.17	10.34	26.50	11.78	14.91	12.19	11.29	2.89
28 g of soy-bean carrier admixed with protease material*	12.6	0.78	0.18	0.23	0.51	0.97	0.69	0.61	0.48	0.31	0.10

* Essential amino acids determined by actual analysis with a Beckman 121 amino acid analyzer.

As will be seen from Table 1, in addition to methionine, corn gluten meal was employed to supplement isolated soybean protein as it has a relatively high content of aromatic amino acids and valine which are relatively lacking in soybean protein.

From Table 4 it will be seen that except for lysine and valine, the 8 remaining essential amino acids were offered to the experimental calves fed 50%P and 80%P in a higher quantity than for the control calves fed 0%P. In the first week, when 500 g of replacer was fed per calf per day (+28 g of soybean carrier admixed with protease material for experimental calves), the lysine deficit for calves fed 50%P was 0.39 g/head (4.6%) and for calves fed 80%P, 0.32 g/head (3.8%). The corresponding deficit for valine was 0.56 g (8.8%) and 0.39 g (6.1%) respectively. When the milk replacer consumption peaked with 900 g consumed/day/calf (+28 g of soybean carrier admixed with protease material for experimental calves), the lysine deficit was 1.33 g (8.7%) and 1.2 g/head (7.9%) respectively and the valine deficit was 1.25 g. (10.9%) and 0.96 g/head (8.4%) respectively.

The feed intake was recorded daily and calves were weighed weekly.

All calves were weaned abruptly after 6 weeks and kept for an additional 2 week period on starter diet and hay.

The performance of the calves up to 6 weeks of age (weaning) is summarised in Table 4.

Table 4

Characteristics	0%P	50%P	80%P
No. of calves	10	10	12
Ave. daily gain (g)	617.9	639.2	710.2 (114.7%) (1)
Milk replacer consumption/kg of gain (kg)	1.22	1.18	1.06 (87%)
Ave. consumption of 18% crude protein calf starter/head/day (g)	399	341	508 (127.3%)
Protein consumption/kg of gain (g)	379.8	374.0	403.1 (106.13%)
Plant protein of total protein (%)	32.5	64.2	85.8
Feed cost/kg of gain (c)	97.5	64.1	51.3
Age at which calves consumed more than 500 g calf starter/day for 3 consecutive days	34 days	36 days	22 days

(1) Values in brackets relate to 0%P as 100%.

From Table 4 it will be seen that the calves receiving the 50%P and 80%P diets had in fact slightly higher average daily gains than those fed on the 0%P replacer.

Calf starter consumption per day up to weaning was 399 g for calves fed 0%P, as against 341 g and 508 g for calves fed 50%P and 80%P respectively, the last value being significantly greater. Calves fed 80%P consumed more than 500 g of starter/head/day for 3 consecutive days after 22 days on experiment. 0%P calves consumed this quantity after 34 days and 50%P after 36 days on experiment. Higher and

earlier intake of solid food by 80%P calves was influenced by earlier involvement of the rumen in food digestion as indicated by regular rumination in the course of the third experimental week.

Hay consumption/calf/day up to weaning was 43.4 g for 0%P, as against 65.1 g and 61.5 g respectively for 50%P and 80%P. Again, more than 40% higher consumption of hay in experimental groups suggests a better functioning rumen.

Cumulative consumption of protein (including starter and hay) /kg of gain for the same period was not significantly different across the whole group. Of the total protein consumed, 32.5% was of plant origin in 0%P versus 64.2% and 85.8% respectively in 50%P and 80%P groups.

No adverse effect was observed on the consistency of the feces in the calves in the 50%P and 80%P groups. Forty-nine occurrences of abnormal feces were observed with the 0%P group as compared with thirty-eight occurrences in the 50%P group and fifteen occurrences in the 80%P group. It was concluded that no serious digestive disturbances occurred in the calves in 50%P and 80%P groups.

EXAMPLE 2

Three groups of calves were raised up to age eight weeks. Apart from points of difference noted below, the procedures of Example 1 were adopted. The first group (5 calves) was fed on the 0%P replacer of Example 1 and the second and third groups (5 and 6 calves, respectively) received the 80%P replacer. Only the second group received a protease material supplement in accordance with this invention.

In this Example, the whole fermentation broth, obtained

as described in Example 1, was concentrated by evaporation, then dialyzed and freeze-dried. The calves in the second group received a dosage of 100,000 D.U. freeze-dried material with each of their twice-daily feedings, providing them with a daily total of 200,000 D.U. of the protease material.

As in Example 1, all calves were weaned abruptly after 6 weeks and kept for an additional 2 week period on starter diet and hay.

At the end of the trial, it was found that the average daily gain was 480.3 g for calves in the first group, 492.8 g for the second group and 450.0 g for the third group. The calf starter consumption/calf/day was 485.9 g for the first group, 573.8 for the second group and 560.0 for the third group.

With the calves in the third group there was a higher occurrence of abnormality in the consistency of the feces than in the calves in the second group (65 occurrences as against 56).

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A feed composition for young calves comprising in combination a milk replacer containing non-milk protein, and a protease material which is an extra-cellular metabolite of a Sorangium sp. (isolate 495) in an amount giving a protease activity of at least 500 D.U. per gram of non-milk protein in the milk replacer.

2. A composition as claimed in claim 1 wherein up to 80% by weight of the total protein in said milk replacer is non-milk protein.

3. A composition as claimed in claim 1 wherein 20 to 80% by weight of the total protein in said milk replacer is non-milk protein.

4. A composition as claimed in claim 1, wherein said non-milk protein is plant protein.

5. A composition as claimed in claim 1, wherein the protein content of said milk replacer comprises 50% by weight non-milk protein.

6. A composition as claimed in claim 1 wherein the protein content of said milk replacer comprises 80% by weight non-milk protein.

7. A composition as claimed in claim 1 wherein said protease material is present in an amount giving a protease activity in the range 500 to 3,000 D.U. per gram of non-milk protein in the milk replacer.

8. A composition as claimed in claim 7 wherein said protease activity is about 2,000 D.U. per gram of non-milk protein in the milk replacer.

9. A composition as claimed in claim 1 wherein said protease material is present in the form of a whole fermentation broth of said Sorangium sp. (isolate 495).

10. A composition as claimed in claim 1 wherein said protease material is present as the isolated liquid portion of a fermentation broth of said Sorangium sp. (isolate 495).

11. A composition as claimed in claim 1 wherein said milk replacer contains a blend of soy protein and corn gluten meal as non-milk protein and contains an addition of methionine giving balanced proportions of essential amino-acids substantially corresponding to those of milk protein.

12. A unit dosage of a feed supplement for young calves containing protease material which is an extra-cellular metabolite of a Sorangium sp. (isolate 495) in an amount exhibiting a protease activity in the range 10,000 to 200,000 D.U.

13. A unit dosage as claimed in claim 12 wherein said protease activity is about 50,000 D.U.

SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente